Newest Kid on the Block: Characterization of the novel multidrug-resistant pathogen, *Candida auris*

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Introduction

Candida auris is a novel and emerging fungal pathogen capable of causing invasive, and often fatal bloodstream and wound infections in immunocompromised patients. Several outbreaks have been reported in hospitals across the world, including the United States, and predominantly in New York. *C. auris* exhibits extensive multidrug resistance that has never been seen in any *Candida* species, and is often misdiagnosed for other *Candida* species, thus limiting treatment options for patients.

Methods

Strains: 10 *C. auris* strains from the Centers for Disease Control, USA, were used in this study and maintained on YPD agar.

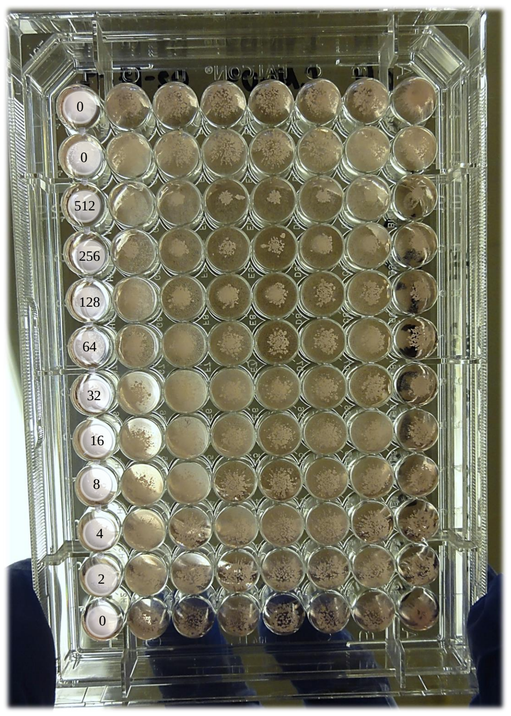
Minimum Inhibitory Concentration: MIC was performed per Clinical and Laboratory Standards Institute guidelines. Strains were suspended in RPMI-MOPS growth media, counted via hemocytometer, and transferred to a 96-well plate and subjected to Fluconazole (2-512 µg/ml) for 48h at 37°C.

Infection studies: *C. auris* was used to infect *Galleria mellonella* as described previously (Cotter et al., 2000). Strains were suspended in YPD broth and CFU was determined via hemocytometer. 10^5 CFU/ larvae or PBS control was injected into worm proleg, and were monitored for 16d. Dead or pupated worms were removed from the experiment.

Macrophage killing assay was performed as previously described, *Bouklas et. al.* 2013.

Results

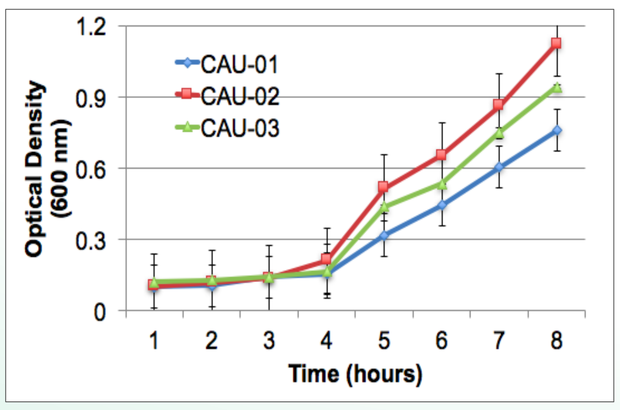
Minimum Inhibitory Concentration using Fluconazole



A representative 96-well plate demonstrating MIC of CAU-03 using serial dilutions of fluconazole. The first concentration exhibiting inhibitory action is 128 µg/mL.

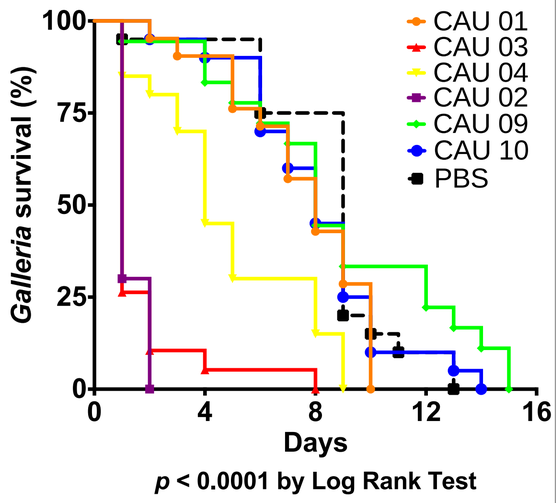
Strain	Minimum Inhibitory Concentration (µg/mL)
CAU-01	4
CAU-02	16
CAU-03	128
CAU-04	128
CAU-05	>256
CAU-06	>512
CAU-07	256
CAU-08	256
CAU-09	>256
CAU-10	256

Growth Curve



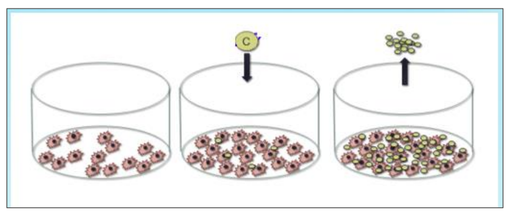
Growth curve of CAU-01, 02, and 03 plotted by Optical Density (600 nm) versus time. All strains exhibited exponential doubling time at approximately 5h.

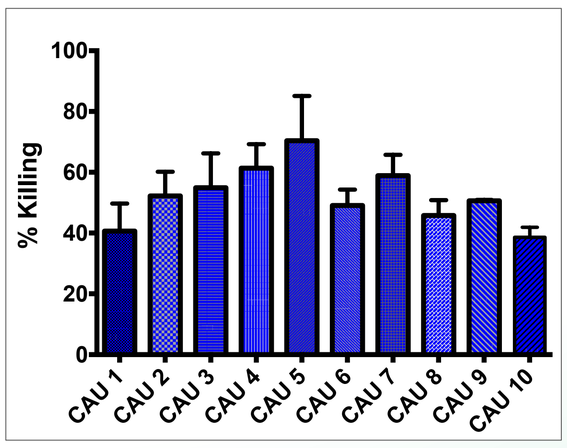
Infection Studies with *Galleria mellonella*



Comparative virulence of C. auris strains in the Galleria host with a PBS control for comparison. Percentage of survival was calculated 16d post-inoculation.

Macrophage Killing Assay





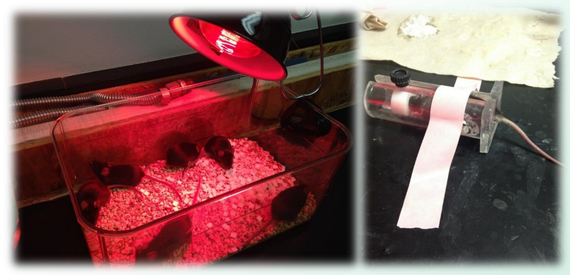
Killing of C. auris strains was determined in LPS and IFN-γ stimulated murine macrophage cell line, J774.16 post 1h of phagocytosis and 1h of killing. 40-60% killing was observed on average, although killing rates were highly variable between strains.

Conclusions

- 70% of the *C. auris* strains tested demonstrate a high drug-resistance (>256 ug/mL) to azoles, specifically fluconazole, due to an as yet undetermined mechanism.
- *C. auris* strains demonstrate variable fitness as observed by their different growth rates with replication times ranging from 30min to 90min.
- *C. auris* strains demonstrate variable virulence in an infection model, which may stem from their response to the hemocyte's phagocytic ability.
- *C. auris* strains are killed variably by murine macrophages, and may therefore invoke different immune responses in a mammalian host.

Future Goals

- Determine the MIC of other antifungal drug classes, such as polyenes and echinocandins.
- Establish lethal dose for mice studies.
- Evaluate drug therapy on infected mice or worms.



Acknowledgments

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